MASTER'S THESIS

Tyrosine hydroxylase-green fluorescence protein transgenic zebrafish as a biosensor and animal model for nicotine and ketamine drug effects

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Tyrosine Hydroxylase-Green Fluorescence Protein Transgenic Zebrafish as A Biosensor and Animal Model for Nicotine and Ketamine Drug Effects

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Abstract

Zebrafish has become a common vertebrate model for study of neurogenesis and neurodevelopment. The transparent rapid development and more close relationship to humans then invertebrate models were the leading reasons for using them in neurological research. Recently, zebrafish has been employed as model to study neurological disorders of addictive drugs by analyzing behavior, morphological and neuroregulatory systems. Taking the advantage of transparent body, transfection of green fluorescent protein (GFP) in zebrafish is developed and widely used to label endogenous protein, cells, organs or even organelles.

In the present study, green fluorescent plasmid constructs were produced containing the promoter of tyrosine hydroxylase (TH; a key synthetic enzyme for catecholamines) and GFP. The constructs were microinjected into zebrafish embryonic cells during the one-cell stage. At 3 days post-fertilization (dpf), GFP started to express in olfactory bulb (OB), telencephalon (Tel), posterior tuberculum (TPp), pretectal area (PPv) and periventricular hypothalamus (PTN) of zebrafish. The present results were confirmed by TH immunohistochemical staining and 6-hydroxydopamine (6-OHDA) challenge in the zebrafish with the same developmental stages. This transgenic fish model provided a novel drug response model which can also be used for studying neurological disorders relating to catecholamines in the nervous system.

Nicotine and ketamine used as a drug in present study to alter intrinsic TH level in zebrafish brain. They had different pharmacological mechanisms that inducing stimulative effects by binding to distinct receptor which further activating the synthesis and release of dopamine. First, locomotion assay was examined to study the general excitatory effects of nicotine and ketamine. Locomotion activities were markedly elevated in a wide range of nicotine concentrations and low doses of ketamine treatment. Since increased locomotion activity was due to activation of dopamine release and excitatory synaptic transmission, it implied that TH level was elevated followed by increase of locomotion activity. Second, TH protein level was assessed in Western blot analysis. Same as the above results, TH protein levels were significantly increased followed by a rising concentrations of nicotine and low doses of ketamine treatments. Finally, TH expression was examined in prior established transgenic zebrafish model. Surprisingly, the trend of TH induction was similar to the results in western blotting.

Based on the parallel results in drug response, TH-GFP transgenic zebrafish model is reliable and useful for expressing intrinsic TH level in a more effective way. The effective transgenic model prevents abundant processes in other experimental assays. TH-GFP transgenic zebrafish, as a novel high throughput sensing model, is highly recommended to be used in drug testing.

Table of contents